MOL231: Investigating the effect of different media and temperatures on oocyte viability

Thea Suul and Kristine Trøgstad
Department of Biological Sciences, University of Bergen

Michael Sars Centre

INTRODUCTION:

Oocytes from *Xenopus laevis* are commonly used as an expression system in research and drug development [(1)]. One of their main advantages as an expression system is their large size, which makes them ideal for RNA injection and electrophysiological recordings[(2)] using two-electrode voltage clamp (TEVC). The oocytes are stored in salt solutions with antibiotics. The oocytes show a decrease in quality and viability after approximately 7 days post-isolation. The decrease in viability is seen in the form of membrane ruptures and no clear separation of animal and vegetal poles[(3)].

Our approach on this study was to observe if it was possible to improve the *Xenopus laevis* oocytes' viability through various media and incubation conditions. Following this approach we wanted to evaluate the oocytes' ability to support RNA injection and recordings using TEVC. Until this project, the oocytes were observed to remain viable at 18°C for about one week in the Lynagh Lab. At the end of the week, the quality and viability of the oocytes were weakened to the point where one could not inject them with RNA. Let alone incubate and perform electrophysiological recordings from them.

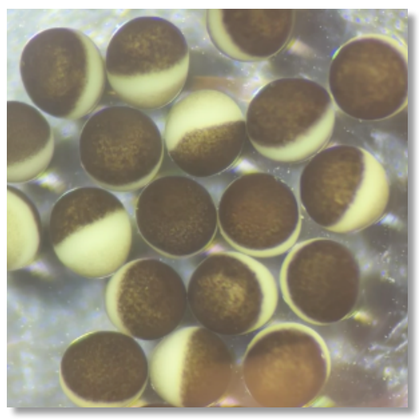
METHODS:

Oocyte storage:
Xenopus laevis oocytes were separated and kept in two different solutions. Modified Barth's solution (MBS) pH at 7.4. The second media was Leibovitz's L-15 Medium (L-15), pH at 7.6. 270 oocytes were sorted into different conditions at different temperatures 4°C, 8°C, or 18°C and for three different projects, 1-week, 2-week, and 3-week. Every 2-3 days half of the media was changed for every condition and optically controlled and documented with microscopy.

Electrophysiology:
The oocytes were injected at different days of incubation with either RNA encoding FMRFa gated sodium channels 50 ng/µL or RatGluD2 20 ng/µL per cell. After RNA injections and the incubation period the oocytes which are viable were recorded from using TEVC.

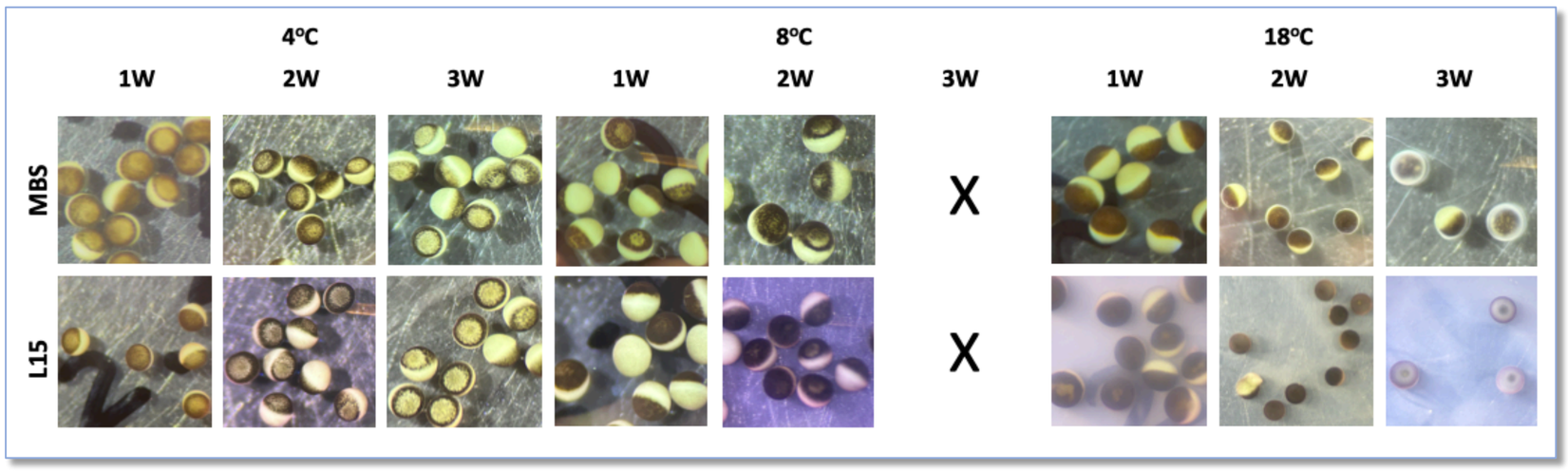
Confocal microscope:
Injected oocytes were fixed, placed in agarose, sectioned, and mounted before being photographed with a confocal microscope.

OOCYTES ON ARRIVAL

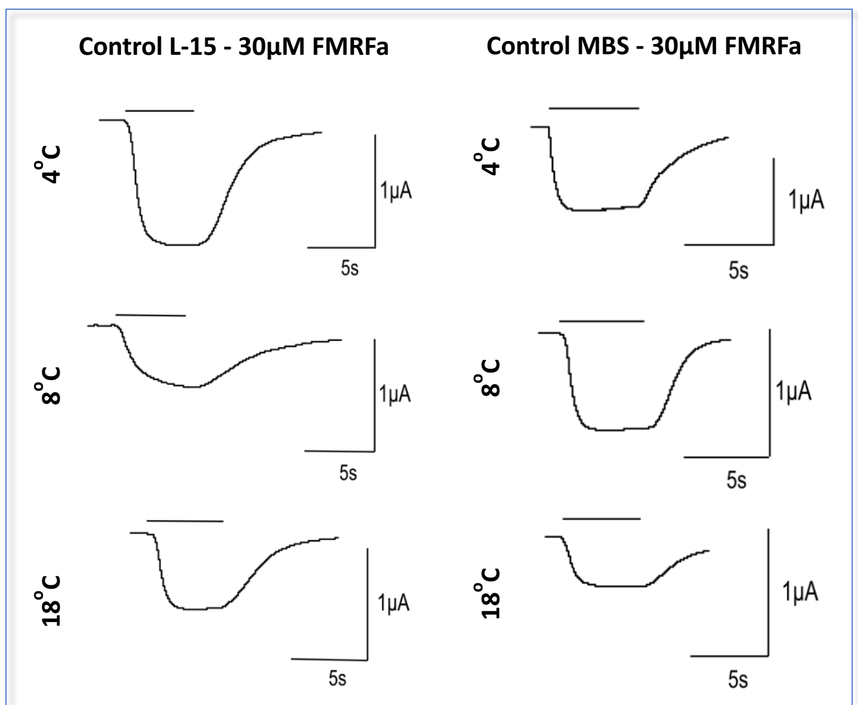


RESULTS:

OOCYTES IN DIFFERENT CONDITIONS



ELECTROPHYSIOLOGY



SURVIVAL RATES

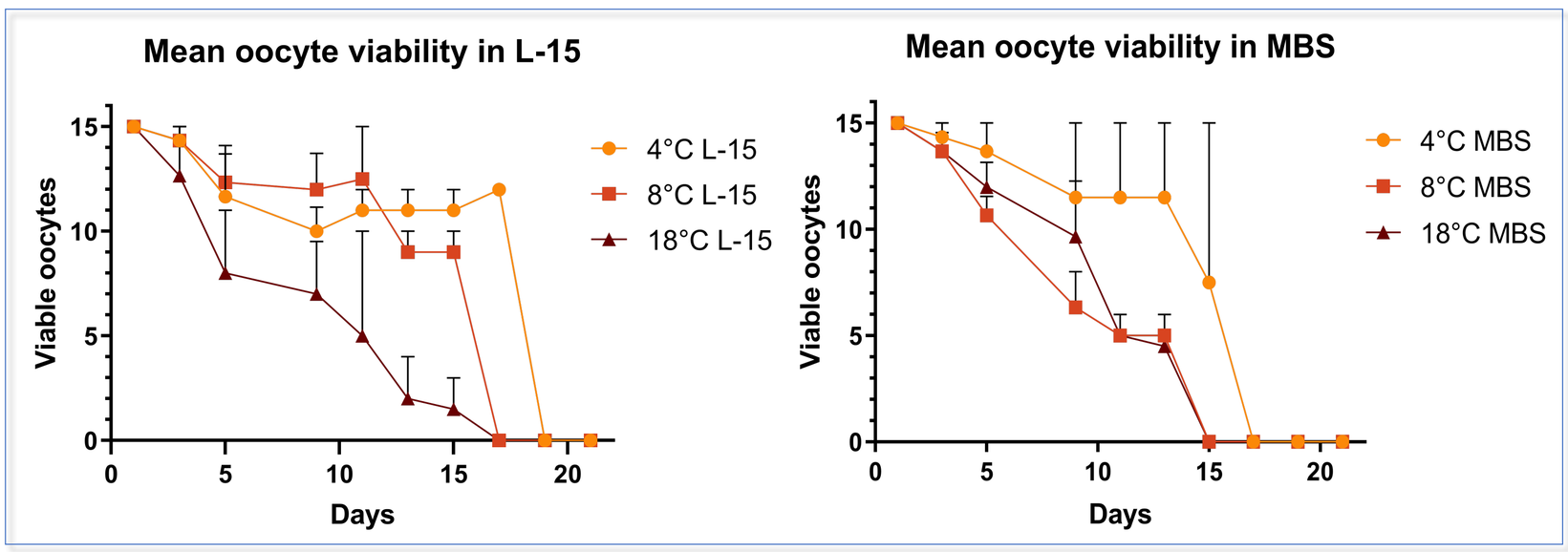
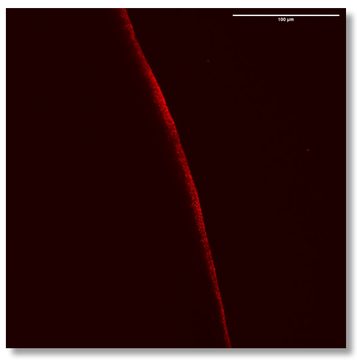


IMAGE CAPTURED WITH CONFOCAL MICROSCOPE



DISCUSSION:

We conducted experiments on oocytes to determine the best way to improve their viability. The results showed that oocytes had longer viability at 4°C and 8°C in both MBS and L-15 media. We determined that MBS seems to be the most suitable media for oocyte viability. However, there was no conclusive evidence that oocytes functioned better after injection. In most of the oocytes that were maintained for 1 week, no currents were observed in response to 30µM FMRFa. However, oocytes injected with the same construct within one week post isolation, exhibited currents in response to 30µM FMRFa. We were not able to measure the currents for the 2- and 3-week projects, as the oocytes disintegrated in the recording chamber. We also observed ruptures in the oocytes when trying to inject them. Further experiments are required to investigate when the oocytes' viability starts to degrade and if storage at lower temperatures affects protein expression. Since the currents were only observed in control oocytes, we proceeded with confocal microscopy using only control oocytes to investigate how viable oocytes express ion channels in the cell membrane. The control oocytes displayed good expression of ion-channels showing that the channels express in the cell membrane in viable oocytes.

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